

## **OPTICAL ALIGNMENT SYSTEM FOR FLOW CYTOMETRY**

This Application is a continuation-in-part application of co-pending U.S. Patent Application Serial No. 10/225,325, filed August 21, 2002, which is a continuation-in-part application of U.S. Patent Application Serial No. 09/630,927, 5 filed August 2, 2000, and entitled "OPTICAL DETECTION SYSTEM FOR FLOW CYTOMETRY", now U.S. Patent No. 6,549,275.

### Background

The present invention relates generally to alignment systems, and more particularly, to optically aligning a light beam with the core flow of a flow stream.

10

### Summary

15

The present invention is directed at an optical alignment system for aligning a light beam with a core flow of a flow stream. A flow stream may include a sheath fluid and a core flow, where the core flow has a current position within the flow stream. A light source may be used to produce a light beam, and an optical element may be used to direct the light beam at the core flow. In some illustrative embodiments, an actuator is provided to move the optical element, light source and/or flow stream such that the light directed by the optical element is aligned with the current position of the core flow.

20

### Brief Description of the Drawings

Other objects of the present invention and many of the attendant advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, in which like reference numerals designate like parts throughout the figures thereof and wherein:

25

Figure 1 is a schematic diagram showing an illustrative embodiment of the present invention;

Figure 2 is a perspective view of an illustrative portable cytometer in accordance with the present invention;

Figure 3 is a schematic view of the illustrative portable cytometer of Figure 2;

Figure 4 is a more detailed schematic diagram showing the portable cytometer of Figure 3 with the cover not yet depressed;

Figure 5 is a more detailed schematic diagram showing the portable cytometer of Figure 3 with the cover depressed;

5 Figure 6 is a schematic diagram showing the formation of a flow stream by the hydrodynamic focusing block 88 of Figure 4;

Figure 7 is a schematic diagram showing an illustrative embodiment of the present invention;

10 Figure 8 is a timing diagram showing an illustrative method for activating the light sources of Figure 7.

Figure 9 is a schematic diagram showing three separate arrays of light sources and detectors, each positioned along a different light source axis relative to the central flow axis of the flow stream of Figure 6;

15 Figure 10 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align the first object relative to the second object;

Figure 11 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light source and/or light detector relative to the second object;

20 Figure 12 is a schematic diagram showing overlapping elongated beam spots provided by an illustrative beam former;

Figure 13 is a graph showing the light illumination intensity for two spaced laser sources, each producing a beam spot having a Gaussian peak light intensity;

25 Figure 14 is a graph showing the light illumination intensity for two spaced laser sources after the light has been provided through a beam former in accordance with the present invention;

Figure 15 is a schematic diagram showing an illustrative beam former for use with a single light source;

Figure 16 is a schematic diagram showing an illustrative beam former for use

with a linear array of light sources;

Figure 17 is a schematic diagram showing a number of illustrative scenarios for detecting the alignment of the cartridge relative to the base and/or cover;

5 Figure 18 is a schematic diagram showing an illustrate method for detecting the alignment of the core flow in the flow channel and for making scatter measurements;

Figure 19 is a schematic diagram of a laminated cartridge having a flow channel 502 and one or more light blocking layers or regions;

10 Figure 20 is a cross-sectional side view of the cartridge of Figure 19;

Figure 21 is a schematic diagram of an illustrative object that has a light scattering element provided thereon or therein;

Figure 22 is a cross-sectional side view of the light scattering element of Figure 21;

15 Figure 23 is a schematic diagram showing an illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream;

Figure 24 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream;

20 Figure 25 is a schematic diagram showing yet another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream;

25 Figure 26 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream; and

Figure 27 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream.

Detailed Description

Figure 1 is a schematic diagram showing an illustrative embodiment of the present invention. The illustrative embodiment includes a first object 2 and a second object 3, wherein the second object 3 includes a slot 4 for receiving the first object 2. While a slot 4 is used in this example, it is not required and some embodiments may 5 not include a slot. The second object 3 shown in Figure 1 includes a linear array of light sources 5a and a linear array of light detectors 6a. While a linear array is used in this example, any suitable array or configuration may be used. Each light source is represented by a plus sign (+) and each detector is represented by a box. The light sources 5a may include, for example, Vertical Cavity Surface Emitting Lasers 10 (VCSELs), edge emitting lasers, Light Emitting Diodes (LEDs), an end of an illuminated optical fiber, or any other suitable light source. The light detectors 6a may include, for example, photo diodes or any other suitable light detector. The detectors 6a may be square, circular, annular or any other suitable shape, as desired. In addition, the detectors 6a may be a single or small number of detectors that detect 15 light from a wide range of locations. In some cases, optics may be used to direct the light from the wide range of locations to the single or small number of detectors, as further described below with respect to Figure 16.

In the embodiment shown, the linear array of light sources 5a are mounted on one side (e.g. upper side) of the slot 4 in the second object 3, and the linear array of 20 light detectors 6a are mounted on an opposite side (e.g., lower side) of the slot 4 of the second object 3. However, in some embodiments, the light sources 5a and the light detectors 6a may be mounted on the same side of the slot 4, such as when the light scattering elements are reflective. The pitch and/or spacing of the linear array of light sources 5a and light detectors 6a may be set to achieve the desired accuracy of 25 alignment detection, as desired.

In Figure 1, the first object 2 includes an elongated light scattering element 7a that extends substantially perpendicular to the linear array of light sources 5a and light detectors 6a when the first object 2 is inserted into the slot 4 of the second object 3. The term “light scattering element”, as used herein, may include any optical element

that diverts, changes, reflects, refracts, absorbs, or otherwise alters a light beam. The one or more light scattering elements 7a may include, for example, one or more lenses, edges or steps, diffraction gratings, absorptive filters, reflectors, flow channels, or any other type of light scattering element. Other portions of the first object 2 may be 5 clear, opaque or substantially non-transparent, as desired.

In the illustrative embodiment shown in Figure 1, each of the light sources 5a is adapted to provide a light beam that is directed toward the slot 4 and to one or more corresponding detectors 6a. The linear array of light sources 5a may be positioned with respect to the slot 4 so that as long as the first object 2 and second object 3 are 10 aligned within a predetermined range 8, one or more of the light beams will intersect at least one of the light scattering elements 7a, which then produces a scattered light profile at one or more of the corresponding detectors 6a. The detectors 6a may be positioned such that at least one of the detectors 6a will detect the scattered light profile. A controller 9 may be used to identify which of the light sources actually 15 produced the detected scattered light profile, and may correlate the location of the identified light source (s) to an alignment position of the first object 2 relative to a second object 3.

During operation, and in one illustrative embodiment, each of the light sources 5a or a sub-set of light sources may be sequentially activated by the controller 9. 20 Depending on the alignment of the first object 2 relative to the second object 3, a particular light source 5a or light sources may produce a light beam that intersects the light scattering element 7a. The light source 5a or light sources that produce the light beam that intersects the light scattering element 7a can be identified by monitoring the output of the corresponding detectors 6a. By only activating one or a sub-set of light 25 sources 5a at any given time, the light source 5a or light sources that produced the light beam that intersects the light scattering element 7a may be more easily identified. However, it is contemplated that all of the light sources may be simultaneously activated and still be within the scope of the present invention. In any event, by knowing which light source 5a or light sources produced the light beam that intersects

the light scattering element 7a, and the location thereof, the alignment of the first object 2 relative to the second object 3 can be determined.

If the light scattering element 7a is uniform along its length in the X-direction (e.g. the left-right direction), the linear array of light sources 5a and the detectors 6a 5 may be used to determine the alignment position of the first object 2 relative to the second object 3 in the Y direction (e.g. the up-down direction in Figure 1). If, however, the light scattering element 7a is not uniform along its length, and adapted to produce a different light scatter profile depending on where the light strikes the light scattering element 7a along its length, the linear array of light sources 5a and the 10 detectors 6a may be used to determine the alignment position of the first object 2 relative to the second object 3 in both the X and Y direction. In this embodiment, the controller 9 may not only identify which of the light sources actually produced the detected scattered light profile to determine the Y position, as described above, but also may correlate the particular light scatter profile that is detected to an X position.

15 Alternatively, or in addition, a second elongated light scattering element 7b may be secured relative to the first object 2. The second elongated light scattering element 7b may extend in the Y direction, with a second linear array of light sources 5b and light detectors 6b extending substantially perpendicular to the second elongated light scattering element 7b. Then, the second linear array of light sources 20 5b and light detectors 6b may be used in conjunction with the second elongated light scattering element 7b to determine the X position of the first object 2 relative to the second object 3. In some embodiments, the second elongated light scattering element 7b may be non-uniform along its length to help also identify the Y position of the first object 2 relative to the second object 3, if desired. If either or both of the first light 25 scattering element 7a and the second light scattering element 7b are non-uniform along their length, some level or redundancy may be provided in the optical alignment detection system.

It is contemplated that the first object 2 and the second object 3 may be any type of objects. In one example, the first object 2 may be a removable media

component such as a removable print cartridge, a removable data storage cartridge such as a removable tape cartridge or removable flash memory cartridge, a removable bio-analysis cartridge or slide or any other form of removable object. The second object may then accept the removable media. Beyond removable media applications, 5 optical fiber alignment applications, component alignment applications, as well as many other applications are also within the scope of the present invention.

Figure 2 shows an illustrative embodiment of the present invention that includes a removable bio-analysis cartridge. Figure 2 is a perspective view of an illustrative portable cytometer 10, which includes a housing 12 and a removable or 10 replaceable cartridge 14. The illustrative housing 12 includes a base 16, a cover 18, and a hinge 20 that attaches the base 16 to the cover 18. The base 16 includes an array of light sources 22, associated optics and the necessary electronics for operation of the cytometer. The cover 12 includes a manual pressurizing element, pressure-chambers with control microvalves, and an array of light detectors 24 with associated optics.

15 The removable cartridge 14 preferably receives a sample fluid via a sample collector port 32. A cap 38 may be used to protect the sample collector port 32 when the removable cartridge 14 is not in use. The removable cartridge 14 preferably performs blood dilution, red cell lysing, and hydrodynamic focusing for core formation. The removable cartridge 14 may be constructed similar to the fluidic 20 circuits available from Micronics Technologies, some of which are fabricated using a laminated structure with etched channels.

25 The removable cartridge 14 is inserted into the housing when the cover 18 is in the open position. The removable cartridge 14 may include holes 26a and 26b for receiving registration pins 28a and 28b in the base 16, which may help provide alignment and coupling between the different parts of the instrument. In some embodiments, the holes 26a and 26b and registration pins 28a and 28b are not required or even desired, and the alignment detection system described herein is used to detect the alignment of the removable cartridge 14 with respect to the base 16 and cover 18. The removable cartridge 14 may also include a transparent flow stream

window 30, which is in alignment with the array of the light sources 22 and light detectors 24, and one or more light scattering elements (not shown). When the cover is moved to the closed position, and the system is pressurized, the cover 18 provides controlled pressures to pressure receiving ports 34a, 34b, and 34c in the removable cartridge 14 via pressure providing ports 36a, 36b and 36c, respectively.

To initiate a test, the cover 18 is lifted and a new cartridge 14 is placed and registered onto the base 16. A blood sample is introduced into the sample collector 32. The cover 18 is closed and the system is manually pressurized. Once pressurized, the instrument performs a white blood cell cytometry measurement. The removable cartridge 14 provides blood dilution, red cell lysing, and hydrodynamic focusing for core formation. The light sources 22, light detectors 24 and associated control and processing electronics perform solid state alignment detection and correction for the particular position of cartridge 14, as well as differentiation and counting of white blood cells based on light scattering signals. Rather than using a hinged construction for the housing 12, it is contemplated that a sliding cartridge slot or any other suitable construction may be used.

Figure 3 is a schematic view of the illustrative portable cytometer of Figure 2. As above, the base 16 may include an array of light sources 22, associated optics and the necessary control and processing electronics 40 for operation of the cytometer. The base 16 may also include a battery 42 for powering the cytometer. The cover 12 is shown having a manual pressurizing element 44, pressure-chambers 46a, 46b and 46c with control microvalves, and light detectors 24 with associated optics.

The removable cartridge 14 may receive a sample fluid via the sample collector port 32. When pressurized by the cover 18, the removable cartridge 14 performs blood dilution, red cell lysing, and hydrodynamic focusing for core formation in a preferred embodiment. Once formed, the core is provided down a flow stream path 50, which passes the flow stream window 30 of Figure 2. The array of light sources 22 and associated optics in the base provide light through the core stream via the flow stream window 30. The detector(s) and associated optics receive

scattered and non-scattered light from the core, also via the flow stream window 30. The controller or processor 40 receives output signals from detector(s), and differentiates and counts selected white blood cells that are present in the core stream.

It is contemplated that the removable cartridge 14 may include a fluid control block 48 for helping control the velocity of each of the fluids. In the illustrative embodiment, the fluid control block 48 includes flow sensors for sensing the velocity of the various fluids and reports the velocities to the controller or processor 40. The controller or processor 40 may then adjust the microvalves associated with pressure-chambers 46a, 46b and 46c to achieve the desired pressures and thus desired fluid velocities for proper operation of the cytometer.

Because blood and other biological waste can spread disease, the removable cartridge 14 preferably has a waste reservoir 52 downstream of the flow stream window 30. The waste reservoir 52 receives and stores the fluid of the flow stream in the removable cartridge 14. When a test is completed, the removable cartridge may be removed and disposed of, preferably in a container compatible with biological waste.

Figure 4 is a more detailed schematic diagram showing the portable cytometer of Figure 3 with the cover 18 not yet depressed. Figure 5 is a more detailed schematic diagram showing the portable cytometer of Figure 3 with the cover depressed. The cover 18 is shown having a manual pressurizing element 44, pressure-chambers 46a, 46b and 46c, and control microvalves generally shown at 60. The array of light sources and detectors are not shown in these Figures.

There are three pressure chambers 46a, 46b and 46c, one for each fluid to be pressurized. In the illustrative embodiment, pressure chamber 46a provides pressure to a blood sample reservoir 62, pressure chamber 46b provides pressure to a lyse reservoir 64, and pressure chamber 46c provides pressure to a sheath reservoir 66. The size and shape of each pressure chamber 46a, 46b and 46c may be tailored to provide the desired pressure characteristics to the corresponding fluid.

Pressure chamber 46a includes a first pressure chamber 70 and a second pressure chamber 72. A first valve 74 is provided between the first pressure chamber

70 and the second pressure chamber 72 for controllably releasing the pressure in the first pressure chamber 70 to a second pressure chamber 72. A second valve 76, in fluid communication with the second pressure chamber 72, controllably vents the pressure in the second pressure chamber 72. Each valve is preferably an array of electrostatically actuated microvalves that are individually addressable and controllable. Pressure chambers 46b and 46c include similar valves to control the pressures applied to the lyse reservoir 64 and sheath reservoir 66, respectively. Alternatively, each valve may be an array of electrostatically actuated microvalves that are pulse modulated with a controllable duty cycle to achieve a controlled "effective" flow or leak rate.

10 The removable cartridge 14 has pressure receiving ports 34a, 34b, and 34c for receiving the controlled pressures from the cover 18. The controlled pressures are provided to the blood reservoir 62, lyse reservoir 64 and sheath reservoir 66, as shown. The lyse reservoir 64 and sheath reservoir 66 are preferably filled before the removable cartridge 14 is shipped for use, while the blood reservoir 62 is filled from sample collector port 32. A blood sample may be provided to the sample collector port 32, and through capillary action, the blood sample is sucked into the blood reservoir 62. Once the blood sample is in the blood reservoir 62, the cover 18 may be closed and the system may be pressurized.

15 20 A flow sensor is provided in-line with each fluid prior to hydrodynamic focussing. Each flow sensor 80, 100 and 102 measures the velocity of the corresponding fluid. The flow sensors are preferably thermal anemometer type flow sensors, and more preferably microbridge type flow sensor. An output signal from each flow sensor 80, 100 and 102 is provided to controller or processor 40.

25 The controller or processor 40 opens the first valve 74 when the velocity of the blood sample drops below a first predetermined value and opens the second valve 76 when the velocity of the blood sample increases above a second predetermined value. Valves 84, 86, 94 and 96 operate in a similar manner to control the velocities of the lyse and sheath fluids.

During operation, and to pressurize the system, the manual pressurizing element 44 is depressed. In the example shown, the manual pressurizing element 44 includes three plungers, with each plunger received within a corresponding one of the first pressure chambers. The plungers create a relatively high non-precision pressure in the first pressure chambers. Lower, controlled pressures are built in the secondary chambers by opening the first valves 70, 84 and 94, which produce a controllable leak into the secondary chambers. If too much pressure builds up in the secondary pressure chambers, the corresponding vent valve 76, 86 and 96 are opened to relieve the pressure.

When closing the cover 18, the normally open first valves 74, 84 and 94 are closed while the vent valves 76, 86 and 96 are open. When a predetermined pressure  $P$  is achieved in the first pressure chambers, the vent valves 76, 86 and 96 are closed, and the first valves 74, 84 and 94 are opened to build a lower pressure  $P'$  in the secondary pressure chambers. The controlled pressure in the secondary pressure chambers provide the necessary pressures to the fluidic circuit of the removable cartridge 14 to produce fluid flow for the blood, lyse and sheath. The velocity of the fluid flow is then measured by the downstream flow sensors 80, 100 and 102. Each flow sensor provides an output signal that is used by the controller or processor 40 to control the operation of the corresponding first valve and vent valve to provide a desired and constant flow rate for each fluid.

Downstream valves generally shown at 110 may also be provided. Controller or processor 40 may close downstream valves 110 until the system is pressurized. This may help prevent the blood, lyse and sheath from flowing into the fluid circuit before the circuit is pressurized. In another embodiment, downstream valves 110 are opened by mechanical action when the cover is closed.

Figure 6 is a schematic diagram showing the formation of a flow stream and core by the hydrodynamic focusing block 88 of Figure 4. The hydrodynamic focusing block 88 receives blood, lyse and sheath at controlled velocities from the fluid driver. The blood is mixed with lyse, causing the red blood cells to be removed. This is often

referred to as red cell lysing. The remaining white blood cells are provided down a central lumen 150, which is surrounded by sheath fluid to produce a flow stream 50. The flow stream 50 includes a core stream 160 surrounded by the sheath fluid 152. The dimensions of the channel are reduced as shown so that the white blood cells 154 and 156 are in single file. The velocity of the sheath fluid is preferably about 9 times that of the core stream 160. However, the velocity of the sheath fluid and core stream 160 preferably remains sufficiently low to maintain laminar flow in the flow channel.

Light emitters 22 and associated optics are preferably provided adjacent one side of the flow stream 50. One or more light detector(s) 24 and associated optics are provided on another side of the flow stream 50 for receiving the light from the light emitters 22 via the flow stream 50. The output signals from the light detector(s) 24 are provided to controller or processor 40, wherein they are analyzed to identify and/or count selected white blood cells in the core stream 160.

Figure 7 is a schematic diagram showing an array of light sources and an array of light detectors for analysis of the core stream 160 of Figure 6, and for identifying the relative alignment position of the cartridge 14 relative to the base 16 and/or cover 18 (see, for example, Figure 2). The light sources are shown as plus (+) signs and the detectors are shown as boxes. In the embodiment shown, the array of light sources is provided adjacent one side of the flow stream 50, such as in or on the base 16, and the array of light detectors is provided adjacent the opposite side of the flow stream, such as in or on the cover 18. Each of the light detectors preferably corresponds to one of the light sources. In some embodiments, only a single or small number of light detectors are provided that are capable of detecting light from a relatively large area, such as the area corresponding to the array of light sources. In the embodiment shown, the array of light sources and the array of light detectors are arranged along a light source axis 200 that is substantially orthogonal to the axis of the flow stream 50. It is contemplated, however, that the array of light sources and the array of light detectors may be arranged along a light source axis that is offset at any angle relative to the axis of the flow stream 50. Although the array of light sources and the array of

light detectors are shown as linear arrays, any suitable arrangement may be used.

The array of light sources is preferably an array of lasers such as Vertical Cavity Surface Emitting Lasers (VCSEL) fabricated on a common substrate. Because of their vertical emission, VCSELs are ideally suited for packaging in compact 5 instruments such as a portable cytometer. Preferably, the VCSELs are “red” VCSELs that operate at wavelengths that are less than the conventional 850 nm, and more preferably in the 670 nm to 780 nm range, but this is not required. Red VCSELs may have a wavelength, power and polarization characteristic that is ideally suited for scatter measurements. It is contemplated, however, that Light Emitting Diodes 10 (LEDs) or any other suitable light source may be used. The light detectors may be, for example, photo diodes or any other suitable light detector. The detectors may be square, circular, annular or any other suitable shape, as desired.

In some embodiments, each of the light sources is adapted to provide a light beam. To identify the relative alignment position of, for example, the cartridge 14 15 relative to the base 16 and/or cover 18 (e.g., see Figure 2), the array of light sources may extend a sufficient range so that one or more of the light beams will intersect at least one of the light scattering element of the cartridge 14. In the illustrative embodiment, the cartridge 14 includes a number of light scattering elements including, for example, cartridge edge 210, flow channel edge 212, and embossed 20 light scattering elements 214. Each of the light scattering elements may produce a scattered light profile.

The detectors may be located such that at least one of the detectors will detect the scattered light profile of at least one of the light scattering elements. A controller may be used to identify which of the light sources actually produced the detected 25 scattered light profile, and to correlate the location of the identified light source(s) to an alignment position of the cartridge 14 relative to the base 16 and/or cover 18.

During operation, and in one illustrative embodiment, each of the light sources or a sub-set of light sources may be sequentially activated. Depending on the alignment of the cartridge 14 to the base 16 and/or cover 18, a particular light source

or light sources may produce a light beam that intersects a light scattering element, such as light scattering element 214. The light source or light sources that produce the light beam that intersects the light scattering element 214 can be identified by monitoring the output of the corresponding detectors. By only activating one or a 5 sub-set of light sources at any given time, the light source or light sources that produced the light beam that intersects the light scattering element 214 may be more easily identified. By knowing which light source or light sources produced the light beam that intersects the light scattering element 214, and the location thereof, the alignment of the cartridge 14 relative to the base 16 and/or cover 18 can be 10 determined.

Figure 8 is a timing diagram showing an illustrative method for activating the light sources of Figure 7. In the illustrative embodiment, each of the light sources is sequentially activated, beginning with the light source 220 which is located at the bottom of the array of light sources shown in Figure 7. The sequential activation of 15 the light sources is shown generally at 218, where the notation V1, V2, etc., corresponds to the activation of VCSEL1 220a, VCSEL2 220b, etc., of Figure 7. The response of the corresponding detectors is shown generally at 224.

When light source 220a is activated, no scattered light profile is detected at the corresponding detectors because, as shown in Figure 7, the cartridge 14 is not situated 20 between light source 220a and the corresponding detectors. While Figure 7 shows three light detectors for each light source, only the left and right detectors may be used for detecting a scattered light profile in some embodiments. Light source 220b may then be activated. When this occurs, the corresponding detectors detect a scatter light profile 222. The characteristics of the scatter light profile 222 may identify the light 25 scattering element as the cartridge edge 210.

When the third and forth light sources are activated, no scattered light profile is detected at the corresponding detectors. When the fifth light source 220c is activated, the corresponding detectors detect a scatter light profile 224. The characteristics of the scatter light profile 224 may identify the light scattering element

as an embossed light scattering element 214. Continuing with the example, when light source 220N is activated, the corresponding detectors detect a scatter light profile 226. The characteristics of the scatter light profile 226 may identify the light scattering element as a fluid channel edge 212. For illustration purposes, the light 5 scatter profiles 222, 224 and 226 are shown as having differing amplitudes. However, it is contemplated that any suitable parameter or characteristic may be used to differentiate between the light scatter profiles, as desired. Alternatively, only the locations of the light scattering elements are identified, and no differentiation between light scattering elements is provided. In some embodiments, only the light scatter 10 profile 224 of the embossed light scattering element 214 may be identified, and the detection of the other light scattering elements may be disregarded.

Once this relative alignment of the cartridge 14 is determined, the present invention may identify which of the one or more light source and/or light detector elements have a location that is adjacent the flow stream 50. For example, in the 15 illustrative embodiment of Figure 7, the present invention may identify light sources 220x, 220y and 220z as having a location that is adjacent the flow stream 50. Depending on the relative alignment of the cartridge 14 and the base 16 and/or cover 18, different light sources and/or light detectors may be selected. For example, if the cartridge 14 were moved up so that light source 220b were positioned above the 20 embossed light scattering element 214, then the three light sources immediately above light source 220c would have a location adjacent the flow stream 50, and would be selected. Once the light sources have been identified and selected, the selected light sources and/or light detectors may be used to, for example, detect one or more parameters and/or characteristics of the flow stream.

25 Figure 9 shows another illustrative embodiment of the present invention. This embodiment includes three separate arrays of light sources and light detectors. While three arrays are shown, it is recognized that any suitable number may be used, depending on the application. In the illustrative embodiment, each array of light sources and light detectors is positioned along a different light source axis relative to

the central flow axis of the flow stream.

A first array of light sources and light detectors is shown at 300. In the illustrative embodiment shown, the light sources and light detectors of the first array 300 are arranged in a linear array along a first light source axis. The array of light 5 detectors is positioned in line with the linear array of light sources. The light sources and light detectors of the first array 300 may be used to measure, for example, the lateral alignment of the cells in the flow stream 50, the particle size, and in some cases, the velocity of the particles. Alternatively, or in addition, the first array of light sources and light detectors 300 may be used to detect the position of a light scattering 10 element, such as light scattering element 312, to help determine the alignment of the cartridge 14 relative to the base 16 and/or cover 18. For example, the light scattering element 312 may produce a light scattering profile that can be detected by one or more corresponding detectors. Once the location of the light scattering element 312 is identified, the alignment of the cartridge 14 relative to the base 16 and/or cover 18 can 15 be determined.

A second array of light sources and light detectors is shown at 302. The second array of light sources may be arranged in a linear array along a second light source axis relative to the flow axis of the flow stream 50. In the illustrative embodiment, the light detectors of the second array 302 include three linear arrays of 20 light detectors. One linear array of light detectors is positioned in line with the linear array of light sources. The other two linear arrays of light detectors are placed on either side of the in-line array of light detectors. The second array of light sources and light detectors 302 is similar that shown and described with respect to Figure 7. As detailed with respect to Figure 7, the second array of light sources and light detectors 25 302 may be used to, for example, help determine the relative alignment of the cartridge 14 with the base 16 and/or cover 18.

Once the relative alignment of the cartridge 14 is determined, one or more light source and/or light detector elements located adjacent the flow stream 50 may be identified. Once these light sources have been identified and selected, the selected

light sources and corresponding light detectors may be used to, for example, detect one or more parameters and/or characteristics of the flow stream. In one illustrative embodiment, the selected light sources and light detectors of the second array 302 may be used to measure the small angle scattering (SALS) produced by selected particles in the flow stream 50. In this case, the outer light detectors may be spaced sufficiently from the in-line detector to intercept the small angle scattering (SALS) produced by selected particles in the flow stream 50.

It is contemplated that the in-line detectors of the second array of light sources and light detectors 302 may be used to detect the light that is not significantly scattered by the particles in the core stream. Thus, the in-line linear array of light detectors of the second array 302 may be used to provide the same measurements as the in-line array of detectors of the first array 300, if desired. The measurements of both in-line arrays of detectors may be compared or combined to provide a more accurate result. Alternatively, or in addition, the in-line detectors of the second array 302 may be used as a redundant set of detectors to improve the reliability of the measurement.

The in-line detectors of the second array 302 may also be used in conjunction with the in-line detectors of the first array 300 to more accurately determine the time-of-flight or velocity of the particles in the flow stream. The measurement may be more accurate because the distance between detectors may be greater. As indicated above, by knowing the velocity of the particles, small variations in the flow rate caused by the fluid driver can be minimized or removed by the controller.

A third array of light sources and light detectors 350 is also shown. The third array of light sources and light detectors 350 may be used to, for example, measure the forward angle scattering (FALS) produced by selected particles in the flow stream. In the illustrative embodiment, the light sources are arranged in a linear array along a third light source axis relative to the flow axis of the flow stream 50. Each light source preferably has a corresponding light detector, and each light detector is preferably annular shaped with a non-sensitive region or a separate in-line detector

positioned in the middle. The annular shaped light detectors may be sized to intercept and detect the forward angle scattering (FALS) produced by selected particles in the flow stream.

5 If a separate in-line detector is provided, it can be used to provide the same measurement as the in-line detectors of the first array 300 and/or second array 302. When so provided, the measurements from all three in-line arrays of detectors of first array 300, second array 302 and third array 350 may be compared or combined to provide an even more accurate result. The in-line detectors of the third array 302 may also be used as another level of redundancy to improve the reliability of the cytometer.

10 The in-line detectors of the third array 350 may also be used in conjunction with the in-line detectors of the first array 300 and/or second array 302 to more accurately determine the time-of-flight or velocity of the particles in the flow stream. The measurement may be more accurate because the distance between detectors may be greater. As indicated above, by knowing the velocity of the particles, small 15 variations in the flow rate caused by the fluid driver can be minimized or removed by the controller.

20 By using three separate arrays of light sources and detectors, and in some embodiments, the optics associated with each array may be optimized for the desired application. For example, and in some embodiments, the optics associated with the first array 300 may be designed to provide well-focused laser light on the plane of the core flow. This may help provide resolution to the alignment, size and particle 25 velocity measurements performed by the first array 300. Likewise, the optics associated with the second array 302 may be designed to provide well-focused laser light on the plane of the core flow. Well focused light is often desirable when measuring the small angle scattering (SALS) produced by selected particles in the flow stream. Finally, the optics associated with the third array 350 may be designed to provide collimated light to the core flow. Collimated light may be desirable when measuring forward angle scattering (FALS) produced by selected particles in the flow stream.

Using arrays of lasers offers a number of important advantages over a single light source configuration. For example, a linear array of lasers may be used to determining the lateral alignment of the path of the particles in the core steam 160. One source of uncertainty in the alignment of the particle stream is the width of the 5 core stream, which leads to statistical fluctuations in the particle path position. These fluctuations can be determined from analysis of the detector data and can be used by the controller or processor 40 to adjust the valves of the fluid driver in order to change the relative pressures that are applied to the sample fluid and the supporting fluids to change the alignment of the selected particles in the flow stream.

10 To determine the lateral alignment of the cells in the fluid stream 50, the cells may pass through several focused spots produced by the array of light sources (e.g. VCSELs). The cells produce a drop in signal in the corresponding in-line reference detectors. The relative strengths of the signals may be used by the controller or processor 40 to determine the center of the particle path and a measure of the particle 15 width.

Another advantage of using an array of light sources rather than a single laser configuration is that the velocity of each cell may be determined. Particle velocity can be an important parameter in estimating the particle size from light scatter signals. In conventional cytometry, the particle velocity is extrapolated from the pump flow rates. 20 A limitation of this approach is that the pumps must be very precise, the tolerance of the cytometer flow chambers must be tightly controlled, no fluid failures such as leaks can occur, and no obstructions such as micro bubbles can be introduced to disturb the flow or core formation.

To determine the velocity of each cell, the system may measure the time 25 required for each cell to pass between two successive spots. For example, and with reference to Figure 9, a cell may pass a detector 208 and then detector 210. By measuring the time required for the cell to travel from detector 208 to detector 210, and by knowing the distance from detector 208 to detector 210, the controller or processor 40 can calculate the velocity of the cell. This would be an approximate

velocity measurement. This is often referred to as a time-of-flight measurement. Once the velocity is known, the time of travel through the spot on which the particle is approximately centered (a few microseconds) may provide a measure of particle length and size.

5 It is contemplated that the particle velocity can also be used to help control the fluid driver. To reduce the size, cost and complexity of a cytometer, the replaceable cartridge 14 of Figure 2 may be manufactured from a plastic laminate or molded parts. While such manufacturing techniques may provide inexpensive parts, they are typically less dimensionally precise and repeatable, with asymmetrical dimensions and wider 10 tolerance cross-sections. These wider tolerances may produce variations in particle velocity, particularly from cartridge to cartridge. To help compensate for these wider tolerances, the time-of-flight measurement discussed above can be used by the controller or processor 40 to adjust the controlled pressures applied to the blood, lyse and sheath 15 fluid streams such that the particles in the core stream have a relatively constant velocity. Also, and because of these wider tolerances, it is often desirable to determine the alignment of the cartridge 14 relative to the base 16 and/or cover 18. Once the alignment position is determined, the appropriate light sources and light detectors can be selected for analyzing the selected parameters or characteristics of the flow stream.

To further evaluate the cell size, it is contemplated that laser beams may be 20 focused both along the cell path and across the cell path. Additionally, multiple samples across the cell may be analyzed for texture features, to correlate morphological features to other cell types. This may provide multiple parameters about cell size that may help separate cell types from one another.

Yet another advantage of using an array of lasers rather than a single laser 25 source configuration is that a relatively constant light illumination may be provided across the flow channel. This may be accomplished by overlapping Gaussian beams provided by adjacent VCSELs, as shown in Figure 12. In single laser systems, the light illumination across the flow channel typically varies across the channel. Thus, if a particle is not in the center of the flow channel, the accuracy of subsequent

measurements may be diminished.

Figure 10 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align the first object relative to the second object. The illustrative embodiment includes a first object 352 and a second object 353, wherein the second object 352 includes a slot 354 for receiving the first object 352. While a slot 354 is used in this example, it is not required and some embodiments may not include a slot. The second object 353 shown in Figure 1 includes one or more light sources, such as light source 355 and one or more light detectors, such as light detector 356.

10 In the embodiment shown, the light source 355 is mounted on one side (e.g. upper side) of the slot 354 in the second object 353, and the light detector 356 is mounted on an opposite side (e.g., lower side) of the slot 354 of the second object 353. Like above, the first object 352 may include an elongated light scattering element 357, as shown.

15 A controller 359 may be used to control a mechanical actuator 361 that, when activated, may move the first object 352 relative to the second object 353. In the embodiment shown, the mechanical actuator 361 moves the first object 352 in an up and/or down direction relative to the second object 353. The actuator 361 may be any type of actuator including, for example, a step motor, a micro actuator such as an 20 electro-statically actuated micro-actuator, or any other suitable actuator, as desired.

During use, the controller 359 may instruct the actuator 361 to move the first object 352 relative to the second object 353 until the light source 355 produces a light beam that intersects the light scattering element 357, which then produces a light scatter profile that can be detected by light detector 356. Once this occurs, the first 25 object 352 may be considered properly aligned with the second object 353. In the illustrative embodiment, the original position of the first object 352 is shown by dotted lines, which is moved in a downward direction until the light scattering element 357 of the first object 352 is aligned with the light source 355. In some embodiments, the light scattering element 357 may be, for example, one more lenses, edges or steps,

diffraction gratings, absorptive filters, reflectors, flow channels, or any other type of light scattering element.

Rather than moving the first object 352 relative to the second object 353, it is contemplated that the light source 355 itself may be moved relative the second object 353. This is illustrated in Figure 11. In Figure 11, an actuator 363 moves the light source 355 relative to the second object 353, which by definition, also moves the light source 355 relative to the first object 352. In the embodiment shown, the controller 359 instructs the actuator 363 to move the light source 355 until the light source 355 produces a light beam that intersects the light scattering element 357 on the first object 352, which then produces a light scatter profile that can be detected by light detector 356. In the illustrative embodiment, the original position of the light source 355 is shown by dotted lines at 370, which after actuation, is moved in a downward direction until the light source 355 is aligned with the scattering element 357 of the first object 352. In some embodiments, a stationary array of light detectors may be used to detect light across a range of locations. In other embodiments, one or more larger stationary detectors may be used to detect light across a range of locations. In still other embodiments, one or more movable light detectors may be used, and moved by the actuator 363 in conjunction with the light source 355, as shown in Figure 11.

Referring now to Figure 12, in some embodiments, the light beams from all or selected light sources may pass through a beam former or the like. When the light sources are in an array that extends along an array axis, the beam former may, for example, increase the beam spot size of each light source in the direction of the axis, and in some cases decrease the beam spot size in a direction perpendicular to the axis. In some embodiments, the beam former may increase the beam spot size in the direction of the axis such that the light output of each light source at least partially overlaps the light output of an adjacent light source. For example, Figure 12 shows a number of beam spots 400a-400f that have been formed by a beam former, wherein each of the beam spots has been increased in the direction of the light source array axis, and decreased in the direction perpendicular to the light source array axis. In

addition, each of the beam spots 400a-400f at least partially overlaps the beam spot of an adjacent light source. This increases the distance that the beam spots 400a-400f can collectively span, and increases the uniformity of light illumination across the illuminated area.

5       Figure 13 shows the light illumination intensity for two spaced laser sources. Each light source produces a beam spot having a Gaussian peak light intensity. A dip in light intensity is shown between the light sources. Figure 14 shows the light illumination intensity for two spaced laser sources after the light has been provided through a beam former as described above. Each of the beam spots has been  
10 increased in the direction of the light source array axis, and decreased in the direction perpendicular to the light source array axis. Also, each of the beam spots at least partially overlaps the beam spot of the adjacent light source. As can be seen, this may increase the uniformity of light illumination across the illuminated area.

15      Figure 15 shows an illustrative beam former that may be used for one or more light sources. The light sources are shown at 410, and may provide a beam spot to a beam former generally shown at 412. The light sources may be, for example, VCSELs, edge emitting photo diodes, or any other suitable light source. The beam former 412 includes a first lens 414 and a second lens 416 that may collectively decrease the beam spot size in the vertical direction, and a third lens 418 that increases  
20 the beam spot size in the horizontal direction. The first lens 414, second lens 416 and the third lens 418 may collectively focus the elongated beam spot 420 on the plane of the core flow 160 of the flow channel 50 in the cartridge 14, as shown. As can be seen, the beam former 412 may increase the distance that the beam spots 420 can span, and may increase the uniformity of light illumination across the flow channel  
25 50. Once the light passes through the core flow 160, the light may be received by another lens (not shown) such as a diffractive optical element (DOE), and may be directed to one or more detectors for detection and analysis.

Figure 16 shows an illustrative beam former for use with a linear array of light sources. The linear array of light sources is generally shown at 450, and may include

a linear array of VCSELs having an array axis that extends in a horizontal direction (X- direction) as shown. A flow channel is shown at 50. The flow channel extends in a vertical direction (Y-direction). One or more detectors is shown at 452. Each of the VCSELs in the array of VCSELs 450 preferably provides a beam spot to beam former 5 456. The beam former 456 may include a number of lenses or other optical elements that collectively form overlapping elongated beam spots, such as those shown in Figure 12. The illustrative beam former 456 may include a first lens 460, a second lens 462 and a third lens 464 that collectively decrease the beam spot size in the vertical direction (Y-direction), and a fourth lens 466 that increases the beam spot size 10 in the horizontal direction. The fourth lens 466 may be, for example, a cylinder lens that is concave in the vertical direction (Y-direction). The first lens 460, second lens 462, third lens 464, and the fourth lens 466 may collectively focus the overlapping elongated beam spots on the plane of the flow channel 50 in the cartridge 14. As detailed with respect to Figure 12, the beam former 456 may increase the distance that 15 the beam spots provided by the array of light sources 450 can collectively span across the cartridge 14, and may increases the uniformity of light illumination across the illuminated area. Once the light passes through the core flow 160, the light may be collected by another lens 470, such as a diffractive optical element (DOE), and may be directed to one or more detector(s) 452 for detection and analysis.

20 Figure 17 is a schematic diagram showing a number of illustrative scenarios for detecting the alignment of the cartridge 14 relative to the base 16 and/or cover 18. To identify the relative alignment position of the cartridge 14 relative to the base 16 and/or cover 18, the array of light sources preferably extend over a sufficient range so that at least one of the elongated beam spots shown in Figure 12 intersects at least one 25 of the light scattering element of the cartridge 14. In the illustrative embodiment shown in Figure 17, the cartridge 14 includes a number of light scattering elements including a cartridge edge 210 and two flow channel edges 212a and 212b. Each of the light scattering elements preferably produces a scattered light profile.

One or more detectors may be located such that at least one of the detectors will detect the scattered light profile of at least one of the light scattering elements. A controller may be used to identify which of the light sources actually produced the detected scattered light profile, and to correlate the location of the identified light source (s) to an alignment position of the cartridge 14 relative to the base 16 and/or cover 18.

In a first scenario, the elongated beam spot region produced by the beam former is collectively shown at 470. In one example, the collective elongated beam spot region 470 is formed by a linear array of ten (10) VCSELs having a 25 micron pitch. The beam former elongates and overlaps the individual beam spots of the 10 VCSEL devices, and produces the collective elongated beam spot region 470 with a length of about 720 microns at the cartridge 14.

In the first scenario, the cartridge 14 is aligned such that the collective elongated beam spot region 470 only overlaps one light scattering element, namely, the cartridge edge 210. If the flow channel 50 were within the range of the 720 micron collective elongated beam spot region 470, the location of the cartridge edge 210 could be used to identify individual VCSELs that are located adjacent the flow channel 50. However, in the embodiment shown, the flow channel 50 is not within the range of the 720 micron collective elongated beam spot region 470. As such, the processor or controller may indicate that the cartridge 14 is misaligned too much to perform an analysis of the flow channel 50. The range covered by the collective elongated beam spot region 470 could be extended by simply adding additional light sources, light detectors and associated optics.

In a second scenario, the cartridge 14 is aligned such that the collective elongated beam spot region 472 overlaps two light scattering elements, namely, the cartridge edge 210 and the flow channel edge 212a. Again, if the entire flow channel 50 were within the range of the 720 micron collective elongated beam spot region 472, the location of the cartridge edge 210 and/or the flow channel edge 212a could be used to identify individual VCSELs that are located adjacent the flow channel 50.

However, in the embodiment shown, the flow channel 50 is not entirely within the range of the 720 micron collective elongated beam spot region 472. As such, the processor or controller may indicate that the cartridge 14 is misaligned too much to perform an analysis of the flow channel 50. The range covered by the collective elongated beam spot region 472 could be extended by simply adding additional light sources and associated optics.

In a third scenario, the cartridge 14 is aligned such that the collective elongated beam spot region 474 overlaps only one light scattering element, namely, the flow channel edge 212a. Again, if the entire flow channel 50 were within the range of the 720 micron collective elongated beam spot region 474, the location of the flow channel edge 212a could be used to identify individual VCSELs that are located adjacent the flow channel 50. However, in the embodiment shown, the flow channel 50 is not entirely within the range of the 720 micron collective elongated beam spot region 474. As such, the processor or controller may indicate that the cartridge 14 is misaligned too much to perform an analysis of the flow channel 50. The range covered by the collective elongated beam spot region 474 could be extended by simply adding additional light sources and associated optics.

In a fourth scenario, the cartridge 14 is aligned such that the collective elongated beam spot region 476 overlaps two light scattering element, namely, the flow channel edge 212a and the flow channel edge 212b. In this scenario, the entire flow channel 50 is within the range of the 720 micron collective elongated beam spot region 476. As such, the location of the flow channel edge 212a and the flow channel edge 212b may be used to identify individual VCSELs that are located adjacent the flow channel 50. Once identified, the identified individual VCSELs may be used to determine selected parameters or characteristics of the flow stream 50.

Figure 18 is a schematic diagram showing an illustrate method for detecting the alignment of the core flow in the flow channel 50 and for making scatter measurements. In the illustrative embodiment, once the VCSELs are identified that are located adjacent the flow channel 50, each of these VCSELs may be sequentially

activated to identify the location of the core in the flow channel 50 and/or to perform scattering measurements, as shown at 480a, 480b and 480c. Alternatively, or in addition, all of the identified VCSELs may be simultaneously activated as shown at 482, and the output of the corresponding detectors may be monitored to determine the 5 location of the core in the flow channel and/or to perform scattering measurements.

Figure 19 is a schematic view of a laminated cartridge 500 having a flow channel 502. Figure 20 is a cross-sectional side view of the cartridge 500 of Figure 19. The cartridge 500 includes a number of laminations, including a bottom lamination 504, a top lamination 506 and one or more intermediate laminations 508. 10 The flow channel 502 may be formed by an etched channel in one or more of the intermediate laminations 508. To help detect a cartridge edge 510, a channel edge 512, or some other feature, one or more light blocking layers or regions may be included in or on one of laminated layers. For example, a light blocking layer or region 514 may be provided on top of the top lamination 506 as shown. The light 15 blocking layer or region 514 may be, for example, a sticker or other filter that is attached to the top and/or bottom surface of the cartridge 500. Alternatively, the light blocking layer may be incorporated into one of the intermediate laminations, as shown at 509, if desired.

The light blocking layer or region may extend, for example, between the 20 cartridge edge 510 and the channel edge 512. The light blocking layer or region 514 may prevent light that is emitted by a light source positioned between the cartridge edge 510 and the channel edge 512 from reaching the corresponding detector(s). This may simplify the detection of the cartridge edge 510 and/or the channel edge 512, because detailed scattering profiles may not need to be analyzed. Instead, a simpler 25 light/no-light algorithm may be used. It is recognized that the light blocking layer or region need not extend between the cartridge edge 510 and the channel edge 512. Rather, it is contemplated that any arrangement suitable for detecting the relative position of the cartridge 500 may be used.

Figure 21 is a schematic diagram of an illustrative object 600 that has a light scattering element 602. Figure 22 is a cross-sectional side view of the light scattering element 602 of Figure 21. A light source 604 (shown as a “+” sign in Figure 21) is shown positioned above the light scattering element 602, and an array of detectors 606 (shown as boxes in Figure 21) are shown positioned below the light scattering element 602. The light source 604 preferably directs a light beam toward the light scattering element 602, and depending the relatively alignment of the light scattering element 602 to the light source 604, the light scattering element 602 may direct the light beam to one or more of the detectors 606. In one example, and referring to Figure 22, if the 10 light source is positioned at position 604a relative to the light scattering element 602, the light scattering element 602 may direct the light beam to detector 606a. If the light source is positioned at position 604b relative to the light scattering element 602, the light scattering element 602 may direct the light beam to a detector 606b. If the light source is positioned at position 604c relative to the light scattering element 602, the 15 light scattering element 602 may direct the light beam to a detector 606c. As such, by monitoring which of the detectors 606 detects the light beam, the relative position of the light source 604 and the light scattering element 602 and thus the object 600 can be determined. In one embodiment, the light scattering element 602 is a lens. However, any suitable light scattering element may be used. It is contemplated that 20 the light scattering element 602 may be used to determine the relative alignment of the object 600 in either one- or two- dimensions.

Figure 23 is a schematic diagram showing an illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream. This illustrative embodiment includes a light source 700 for 25 producing a light beam 702, an optical element 704 for focusing the light beam 702 on the core flow 706 of a flow stream, and a detector 708 for detecting scattered and/or reflected light 710 from the core flow 706. The optical element 704 is shown schematically as a lens, but it may include a set of lenses or any other suitable optical element, as desired. It is also contemplated that another optical element (not shown in

Figure 23) may be provided between the core flow 706 and the detector 708 in some cases, as shown in for example Figure 25-27. Also, it is contemplated that the detector 708 may be position on the same side as the light source, if desired.

5 The core flow 706 is included in a flow stream traveling down a flow channel 712. The flow channel 712 shown in Figure 23 is flowing into the page. The core flow 706 may include a sheath fluid (liquid or gas) flowing on either side of the core flow 706. In some embodiments, the sheath fluid and core flow 706 have laminar flow as they pass through the flow channel 712.

10 As shown generally at 720, the core flow 706 may be relatively centered in the flow channel 712. However, under some conditions, the core flow 706 may not flow down the center or at some other predetermined position in the flow channel 712. For example, as shown generally at 722, the core flow 706 may flow left of center of the flow channel 712. Likewise, as shown generally at 724, the core flow 706 may flow right of center of the flow channel 712.

15 To help compensate for the various possible positions of the core flow 706 within the flow channel 712, it is contemplated that an actuator 726 or the like may be used to move the optical element 704 so that the light beam 702 emitted by the light source 700 is aligned with (e.g. focused on) the current position of the core flow 706 in the flow channel 712. The actuator 726 may be controlled by a controller 728. In 20 some cases, the controller 728 may receive one or more feedback signals indicating whether the light beam 702 is currently aligned with (e.g. focused on) the current position of the core flow 706 in the flow channel 712. If not, the controller 728 may instruct the actuator to move the optical element 704 until the light beam 702 is aligned with (e.g. focused on) the current position of the core flow 706 in the flow 25 channel 712. The feedback signal may include, for example, an output signal from the detector 708.

In one example, and as generally shown at 722, when the core flow 706 is left of center of the flow channel 712, the controller 728 may instruct the actuator 700 to move the optical element 704 to the left, which may direct the light beam 702 at the

current position of the core flow 706 in the flow channel 712. Likewise, and as generally shown at 724, when the core flow 706 is right of center of the flow channel 712, the controller 728 may instruct the actuator 700 to move the optical element 704 to the right, which may direct the light beam 702 at the current position of the core flow 706 in the flow channel 712. In some cases, the controller 728 may instruct the actuator 700 to first move the optical element 704 to identify an edge of the flow channel 712. This may be considered a coarse alignment. In some cases, the flow channel 712 is part of a fluidic cartridge, and the fluidic cartridge is non-transparent except at the flow channel. Thus, as the light beam 702 is directed across an edge of the flow channel 712, an abrupt change in light intensity at the detector may occur. Then, the controller 728 may instruct the actuator 700 to move the optical element 704 to direct the light beam 702 at the current position of the core flow 706 in the flow channel 712.

The actuator 726 may be any type of mechanical actuator. In some cases, the actuator 726 may be a stepper motor, a voice coil, an electrostatic actuator, a magnetic actuator, a micro-positioning actuator similar to that shown and described in U.S. Patent No. 6,445,514, or any other suitable actuator, as desired.

In some embodiments, the light source 700 may include a single light source. In other embodiments, the light source may include more than one light source, such as an array of light sources. In some cases, and when the light source shown at 700 includes more than one light source, at least some of the light sources may produce different wavelengths of light, if desired. The different wavelengths of light may be emitted and imaged onto the core flow by the optical element, as discussed above. Providing multiple wavelengths may be particularly beneficial when exciting fluorescence in at least some of the particles in the core flow, and detecting the fluorescence with the detector. Other applications may also benefit from a multiple wavelength light source.

Figure 24 is similar to the illustrative embodiment shown in Figure 23, but further shows a second optical element 730 between the movable optical element 704

and the flow stream 712. Optical element 730 may be adapted to, for example, help columnate the light beam 702 before it engages the core flow 706, regardless of the incident angle of the light beam 702. In some cases, this may help maintain a more consistent incident light beam on the core flow 706 regardless of the position of the 5 core flow 706 in the flow channel 712.

Figure 25 is a schematic diagram showing yet another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream. This illustrative embodiment includes a light source 750 for producing a light beam 752, a first optical element 754 for focusing the light beam 752 on the core flow 756 of a flow stream, and a detector 758 for detecting scattered light 760 from the core flow 756. In Figure 25, a second optical element 762 is provided between the core flow 756 and the detector 758, but this is not required. The optical elements 754 and 762 are shown schematically as lenses, but they may each include a single lens, a set of lenses, or any other suitable optical element, as 10 15 desired.

As in Figures 23-24, the core flow 756 is included in a flow stream traveling down a flow channel 764. The flow channel 764 shown in Figure 25 is flowing into the page. The core flow 756 may include a sheath fluid (liquid or gas) flowing on either side of the core flow 756. In some embodiments, the sheath fluid and core flow 20 25 756 have laminar flow as they pass through the flow channel 764.

As shown generally at 770, the core flow 756 may be relatively centered in the flow channel 764. However, under some conditions, the core flow 756 may not flow down the center or at some other predetermined position in the flow channel 764. For example, as shown generally at 772, the core flow 756 may flow right of center of the flow channel 764. Likewise, although not shown, the core flow 756 may also flow left of center of the flow channel 764.

To help compensate for the various possible positions of the core flow 756 within the flow channel 764, it is contemplated that an actuator or the like (not explicitly shown in Figure 25) may be used to move the optical element 754 and light,

source 750, generally shown at 774, so that the light beam 752 emitted by the light source 750 is aligned with (e.g. focused on) the current position of the core flow 756 in the flow channel 764. As in Figures 23-24, the actuator may be controlled by a controller. In some cases, the controller may receive one or more feedback signals 5 indicating whether the light beam 752 is currently aligned with (e.g. focused on) the current position of the core flow 756 in the flow channel 764. If not, and as shown generally at 772, the controller may instruct the actuator to move the optical element 754 and the light source 750 until the light beam 752 is aligned with (e.g. focused on) the current position of the core flow 756 in the flow channel 764.

10 Again, the actuator may be any type of mechanical actuator. In some cases, the actuator may be a stepper motor, a voice coil, an electrostatic actuator, a magnetic actuator, a micro-positioning actuator similar to that shown and described in U.S. Patent No. 6,445,514, or any other suitable actuator, as desired.

15 Figure 26 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the core flow of a flow stream. This illustrative embodiment includes a light source 780 for producing a light beam 782, a first optical element 784 for focusing the light beam 782 on the core flow 786 of a flow stream, and a detector 788 for detecting scattered light 790 from the core flow 786. In Figure 26, a second optical element 792 is 20 provided between the core flow 786 and the detector 788, but this is not required. The optical elements 784 and 792 are shown schematically as lenses, but they may each include a single lens, a set of lenses, or any other suitable optical element, as desired.

25 The core flow 786 is included in a flow stream traveling down a flow channel 794. In one illustrative embodiment, the flow channel 794 may be part of, for example, a fluidic cartridge 800. The flow channel 794 shown in Figure 26 is flowing into the page. The core flow 786 may include a sheath fluid (liquid or gas) flowing on either side of the core flow 786. In some embodiments, the sheath fluid and core flow 786 have laminar flow as they pass through the flow channel 794.

As shown generally at 802, the core flow 786 may be relatively centered in the

flow channel 794. However, under some conditions, the core flow 786 may not flow down the center or at some other predetermined position in the flow channel 794. For example, as shown generally at 804, the core flow 786 may flow left of center of the flow channel 794. Likewise, although not shown, the core flow 786 may also flow 5 right of center of the flow channel 794.

To help compensate for the various possible positions of the core flow 786 within the flow channel 794, it is contemplated that an actuator or the like (not explicitly shown in Figure 26) may be used to move the flow channel 794, or in some cases the entire fluidic cartridge 800, so that the light beam 782 emitted by the light 10 source 780 is aligned with (e.g. focused on) the current position of the core flow 786 in the flow channel 794. As detailed above, the actuator may be controlled by a controller. In some cases, the controller may receive one or more feedback signals indicating whether the light beam 782 is currently aligned with (e.g. focused on) the current position of the core flow 786 in the flow channel 794. If not, and as shown 15 generally at 804, the controller may instruct the actuator to move the flow channel 794, or in some cases the entire fluidic cartridge 800, until the light beam 782 is aligned with (e.g. focused on) the current position of the core flow 786 in the flow channel 794.

Again, the actuator may be any type of mechanical actuator. In some cases, 20 the actuator may be a stepper motor, a voice coil, an electrostatic actuator, a magnetic actuator, a micro-positioning actuator similar to that shown and described in U.S. Patent No. 6,445,514, or any other suitable actuator, as desired.

Figure 27 is a schematic diagram showing another illustrative embodiment of the present invention which uses a mechanical actuator to align a light beam with the 25 core flow of a flow stream. This illustrative embodiment includes a light source 900 for producing a light beam 902, a first optical element 904 for focusing the light beam 902 on the core flow (not explicitly shown in Figure 27) in a flow channel 906, a second optical element 908 for focusing scattered light on a detector 910. The illustrative embodiment shown in Figure 27 is similar to that shown in Figure 16.

However, in some embodiments, the light source 902 in Figure 27 may include a single light source rather than an array of light sources.

The core flow is included in a flow stream traveling along a flow channel 906. The flow channel 906 shown in Figure 27 is flowing in an upward direction. The 5 core flow may include a sheath fluid (liquid or gas) flowing on either side of the core. In some embodiments, the sheath fluid and core flow have laminar flow as they pass through the flow channel 906.

As detailed above, the core flow may be relatively centered in the flow channel 906. However, under some conditions, the core flow may not flow down the center or 10 at some other predetermined position in the flow channel 906. For example, in the illustrative embodiment of Figure 27, the core flow may flow left of center or right of center of the flow channel 906.

To help compensate for the various possible positions of the core flow within the flow channel 906, it is contemplated that an actuator or the like (not explicitly 15 shown in Figure 27) may be used to move the optical element 904, as shown by dashed arrows 920a and 920b, so that the light beam 902 emitted by the light source 900 is aligned with (e.g. focused on) the current position of the core flow in the flow channel 906. The actuator may be controlled by a controller. In some cases, the controller may receive one or more feedback signals indicating whether the light beam 20 902 is currently aligned with (e.g. focused on) the current position of the core flow in the flow channel 906. If not, the controller may instruct the actuator to move the optical element 904 until the light beam 902 is aligned with (e.g. focused on) the current position of the core flow in the flow channel 906.

Like above, the actuator may be any type of mechanical actuator. In some 25 cases, the actuator may be a stepper motor, a voice coil, an electrostatic actuator, a magnetic actuator, a micro-positioning actuator similar to that shown and described in U.S. Patent No. 6,445,514, or any other suitable actuator, as desired.

Having thus described the preferred embodiments of the present invention, those of skill in the art will readily appreciate that the teachings found herein may be

applied to yet other embodiments within the scope of the claims hereto attached.